Reference Arkema Inc. and Chevron Phillips Chemical Company LP (2005) Reproduction/developmental toxicity screening test by oral route (gavage) in rats with methanesulfonic acid. CIT report no. 26749 RSR.

Type : Reproduction/developmental toxicity screening test (OECD422)

Test condition :
ANIMALS
- Number: 12 males and 12 females per dose
- Strain : Sprague-Dawley Crl CD (SD) IGS BR
- Breeder: Charles River Laboratories France, L'Arbresle, France
- Age at the beginning of the treatment period: 10 weeks old
- Weight at the beginning of the treatment period: 408 g (range: 350 g to 441 g) for the males and 241 g (range: 211g to 267 g) for the females
- Acclimation: 11-days before the beginning of the treatment period

ENVIRONMENTAL CONDITIONS
- Temperature : 22 ± 2°C
- Relative humidity : 50 ± 20%
- Light/dark cycle : 12h/12h (7:00 - 19:00)
- Ventilation : about 12 cycles/hour of filtered, non-recycled air.

HOUSING
The animals were housed individually in polycarbonate cages or in suspended wire-wesh cages. Autoclaved wood shavings were provided as nesting material, a few days before delivery and during the lactation period

FOOD and WATER
- Food: A04 C pelleted maintenance diet ad libitum
- Water: filtered '0.22 µm filter) tap water ad libitum

TREATMENT
- Vehicle: purified water, obtained by reverse osmosis
- Dosage form preparation: solution in the vehicle at 25, 50 and 100 mg/mL, expressed as active substance. The dosage forms were adjusted to a pH of 7.0 with NaOH
- Volume: 10 ml/kg
- Chemical analysis of the dosage forms:
  . Stability: Two dosage forms (25 and 100 mg/mL) were sampled after 0, 4 and 9 days storage at +4°C
  . Concentration: On weeks 1, 4 and 8

Test substance : Test article name: Methanesulfonic acid
CAS no. : 75-75-2
Source: ARKEMA
Batch no. : 4810A
Purity: 70.50% in water

Route of admin. : gavage

Exposure period : in males: during 4w before mating, the mating period (2w) and until sacrifice.
in females: during 4w before mating, the mating period (2w), pregnancy (3w), lactation until day 4 pp inclusive, and until sacrifice (D5 pp)

Frequency of treatment : 7 days per week

Control group : concurrent vehicle

Method :
CLINICAL EXAMINATIONS
- Morbidity and mortality: at least twice a day
- Clinical signs: at least once a day
- Body weight:
  males: on day 1, then once a week until sacrifice
  females: on day 1, then once a week until mated, then on

days 0, 7, 14 and 20 pc and on days 1 and 4 pp.
- Food consumption
males: once a week (except during the mating period) until sacrifice
females: once a week during the premating period and then on the following intervals: days 0-7, 7-14, 14-20 post-coitum, and days 1-4 post-partum.
MATING
- Mating procedure: one female was placed with one male from the same dose-level group
PARTURITION
Females were allowed to drop their litters normally and rear their progeny until day 4 post partum
OBSERVATIONS OF THE PROGENY DURING THE POST-PARTUM PERIOD
- Litter size: total litter size and numbers of pups of each sex were recorded as soon as possible after birth. The litters were observed daily.
- Clinical signs: daily
- Body weight: days 1 and 4 pp
PATHOLOGY
- Sacrifice
. males: after the end of the mating period,
. females: on day 5 post-partum,
. pups: on day 5 post-partum.
- Organ weights: testes and epididymides
- Macroscopic post-mortem examination: on all parent animals. In all females, the number of implantation sites and corpora lutea were recorded.
- Pups: gross external examination before sacrifice
- Preservation of tissues: ovaries, prostate, seminal vesicles, uterus (horns and cervix), vagina, in 10% buffered formalin. Testes and epididymides, in Bouin's fluid
- Microscopic examination: ovaries, testes and epididymides (with special emphasis on stages of spermatogenesis and histopathology of interstitial testicular cell structure) of all males and females in the control and high-dose groups.

Result : CHEMICAL ANALYSES OF THE DOSAGE FORMS
- Stability: satisfactory stability of the 25 and 100 mg/mL solutions over a 9-day period at +4°C
- Concentration: satisfactory agreement (± 10%) between the nominal and actual concentrations
CLINICAL EXAMINATIONS
- Mortality
Males: no deaths in the control group or in the 250 or 500 mg/kg/day groups. At 1000 mg/kg/day, one male on day 33. No major factor contributing to death was established.
Females: no deaths in any group during the premating period. One female given 250 mg/kg/day was found dead on day 10 post-coitum. This death was consequently considered to be accidental and not related to the treatment.
- Clinical signs
Males: no clinical signs at 250 and 500 mg/kg/day. At 1000 mg/kg/day, ptyalism was transiently observed in 4/11 males from days 20 or 21 of dosing.
Females: no clinical signs at 500 mg/kg/day during the pre-mating and pregnancy periods. At 250 mg/kg/day, regurgitation was observed during the pre-mating period in two females from days 22 to 24 or days 26 to 27, and in three other females for 1 or 3 consecutive days of the

pregnancy period. This sign was not dose-related and was therefore considered to be caused by difficult gavage. At 1000 mg/kg/day, ptyalism was observed in 3/12 females on day 20 of the pre-mating period.
The ptyalism (excess of salivation), noted in four males and three females given 1000 mg/kg/day, is commonly noted following gavage and is not considered as an adverse effect. There were no clinical signs at any dose-level during the lactation period.
- Body weight
    Males: no effect related to treatment
    Females: no effect related to treatment
- Food consumption: no treatment-related effect in males and females
MATING AND FERTILITY DATA
- Mating index: no treatment-related effect
- Pre-coital interval: no treatment-related effect
- Fertility index: no treatment-related effect
- Duration of gestation : no treatment-related effect
- Delivery data: no treatment-related effect
- Post-natal and neo-natal losses: no treatment-related effect
OBSERVATION OF THE PUPS AT BIRTH
- Mean number of liveborn pups per litter: no treatment-related effect
Macroscopic observation: no treatment-related effect
OBSERVATION OF THE PUPS AFTER BIRTH
- Mortality: no treatment-related effect
- Clinical signs: no treatment-related effect
- Pup body weight: no treatment-related effect
- Sex ratio: no treatment-related effect
PATHOLOGY
- Organ weights :
Minor differences, considered to be of no toxicological significance, were observed in the weights of testes and epididymides between treated and control males.
- Macroscopic post-mortem examination : no treatment-related effect
- Microscopic examination:
    Testicular Staging: No treatment-related changes were observed.
    Ovaries: No treatment-related changes were observed. Semi-quantitative evaluation of the morphological characteristics of ovarian physiology:

Dose-level (mg/kg/day)	0	1000
Evaluation of ovarian follicles		
Very few	3/12	5/12
Few	6/12	6/12
moderate	3/12	1/12
Evaluation of corpora lutea		
Few	7/12	6/12
moderate	5/12	4/12
marked	0/12	2/12
Evaluation of follicular atresia		
Very few	2/12	4/12
Few	6/12	8/12
moderate	4/12	0/12

As no major difference was observed between the treated and control groups, it was concluded that there was no effect of treatment by the test item on the ovarian functions.

Conclusion	:	The oral administration of methane sulfonic acid at 250, 500 or 1000 mg/kg/day to male and female Sprague Dawley rats, was well tolerated at all dose-levels. There were no substance-induced effects on the male and female reproductive performance, nor on the progeny of the parental rats up to 1000 mg/kg/day. The no observed effect level (NOEL) for parental toxicity and for toxic effect on reproductive performance and on progeny is 1000 mg/kg/day.